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Renomedullary and intestinal hyaluronan content during body water excess: a study in rats and gerbils

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Our previous studies in rats have suggested a role for renomedullary hyaluronan (HA) in water homeostasis. The gerbil is known for its unique ability to conserve water. In the present study renal papillary and intestinal HA were compared between groups of anaesthetized gerbils and rats before and after up to 6 h of I.V. water loading. Baseline papillary HA in gerbils was only 37 % of that in the rat. Water loading in rats increased the papillary HA content. Elevation was maximal (+27 %, $P < 0.05$) after 2 h of water loading and then declined to control levels after 6 h of water loading (+3 %, n.s.). In contrast, the gerbil responded with a decreased papillary HA content during water loading. The depression was maximal after 2 h (−49 %, $P < 0.05$) and was still 41 % below the control values after 6 h ($P < 0.05$). The urine flow rate increased rapidly in the rat and its maximum, 21 times above the control level ($P < 0.05$), occurred at the HA peak, i.e. after 2 h of water loading while in the gerbil, the urine flow rate increased slowly and slightly and was only six times above control values after 6 h of water loading ($P < 0.05$). The HA content along the intestine was similar in the two species: lowest in the duodenum and jejunum and highest in the distal colon. To conclude, in the rat, the elevation of papillary interstitial HA during acute water loading would counteract water reabsorption by changing the physico-chemical characteristics of the interstitial matrix favouring rapid water diuresis. This would work as a complement to the powerful regulation by ADH. The gerbil has a diametrically different regulation of papillary HA turnover during water loading. The decreased papillary HA level during water loading and the slow and small diuretic response may represent a genetic difference in adaptation to enhance the ability to conserve water in an arid environment.

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Hyaluronan (HA) is a negatively charged glycosaminoglycan made up of repeated disaccharide units consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine (Comper & Laurent, 1978). HA is found in the extracellular matrix where it can form highly viscous solutions (Fraser & Laurent, 1989). HA is an important constituent of the loose connective tissue and is involved in several biological processes, including wound healing, modulation of the inflammatory reaction and maintenance of water and protein homeostasis (Comper & Laurent 1978; Gerdin & Hällgren, 1997).

The amount of HA varies considerably, not only in different organs and organ structures but also during embryonic development and pathological conditions (Gerdin & Hällgren, 1997). In the kidney this affects several conditions such as renal transplant rejection (Hällgren *et al.* 1990) and renal ischaemic damage (Johnsson *et al.* 1996; Lewington *et al.* 2000). The distribution of HA in the healthy rat kidney is heterogeneous, with high concentration in the renal papilla, while very small amounts of HA are found in the cortex (Hällgren *et al.* 1990; Johnsson *et al.* 1996). This high concentration in the inner parts of the kidney, i.e. the medulla, is believed to provide mechanical support for the tubules and blood vessels and also to play a role in renal water handling (Ginetzinsky, 1958; Law & Rowen, 1981; Hansell *et al.* 2000). HA shows a unique water-binding capacity in that 1 g of HA has the ability to bind more than 1 l of water (Laurent & Fraser, 1986), which may influence water transport in the renal medulla and be of importance for maintaining the concentration gradient.

In our previous study in rats (Hansell *et al.* 2000), we found that renal papillary interstitial HA increased during acute water loading and decreased during dehydration. We suggested that an elevated papillary interstitial HA might antagonize water reabsorption, thereby making an important contribution to the diuretic response. We verified our results in an *in vitro* study (Göransson *et al.* 2001) showing that renomedullary interstitial cells produce more HA in a low osmolality medium and less HA

with increasing medium osmolality. Furthermore, in the *in vivo* study (Hansell *et al.* 2000), we also demonstrated that the renal papillary HA content in the gerbil is only about 25 % of that in the rat. The gerbil is a desert rodent, adapted to an arid environment and a number of studies show that several factors influencing the water balance differ between rats and gerbils. These factors reside both in the kidney and brain (Buchanan & Stewart, 1974; Natochin *et al.*, 1983; Wu & Shen, 1994). For example, the papilla of the gerbil is longer than that of the rat, meaning that the vasa recta and loops of Henle are longer. This leads to an increased ability to build a concentration gradient, which is needed for the formation of concentrated urine. The regulation of anti-diuretic hormone (ADH/vasopressin) and the distribution of vasopressinergic neurons (Edwards, 1984; Wu & Shen, 1994) are other factors which differ between the species.

A possible consequence of the low papillary HA content in the normal gerbil could thus be a facilitated water reabsorption to ensure water conservation. The aim of the present study was to compare the response of water loading on renomedullary HA regulation in gerbils and rats. Furthermore, water and electrolytes gain entrance into the systemic circulation via transport across the intestinal epithelium. In fact, the intestine absorbs large amounts of fluid every day, up to 10 l in man. Therefore, it was also interesting to compare gerbils and rats with regard to the HA levels along the intestine.

METHODS

The local Ethics Committee for Animal Experimentation approved the experiments. Forty-five male gerbils (*Meriones unguiculatus*, bred at the Laboratory Animal Department, Biomedical Centre, Uppsala, Sweden) weighing 75 ± 1 g and 43 male Sprague-Dawley rats (B&K, Sollentuna, Sweden) weighing 307 ± 8 g were used. All animals had free access to tap water and a standardized chow (R3, Ewos, Södertälje, Sweden) containing 0.3 % sodium, 0.8 % potassium and 21 % protein. Anaesthesia was induced by an intraperitoneal injection of Inactin (5-ethyl-5-(1-methyl-propyl)-2-thio-barbiturate sodium; Byk-Gulden, Konstanz, Germany), 120 mg (kg body weight)$^{-1}$. The depth of the anaesthesia was tested by pinching the tail and if necessary more Inactin was given. Most of the time no additional anaesthetic was needed. The animals were placed on a servo-controlled heating pad to maintain a rectal temperature of 37.5 °C.

Surgery

Tracheotomy was performed. Thereafter polyethylene cannulas were inserted into the right femoral vein and artery, the former for infusion of isotonic saline (0.9 % NaCl at 0.15 ml (100 g body weight)$^{-1}$ h^{-1} or hypotonic glucose–saline, approximately 100 mosmol (kg H_2O)$^{-1}$ (0.25 % NaCl, 0.5 % glucose at 1.5 ml (100 g body weight)$^{-1}$ h^{-1}) and the latter for continuous measurement of arterial blood pressure. The urinary bladder was catheterized through a suprapubic incision for urine sampling. After the experiment described below had been carried out, the animals were killed with an intravenous injection of saturated KCl.

Protocol

Control. Animals (rats n = 14; gerbils n = 14) received isotonic saline throughout the experiment. Urine was collected during four consecutive 30 min sampling periods. After this, the kidneys and intestines were excised, weighed and sectioned for quantitative HA analysis or histochemical staining for HA as described below.

Water diuresis 2 h. Animals (rats n = 13; gerbils n = 14) received hypotonic glucose–saline solution after surgery. Urine was collected during four consecutive 30 min sampling periods. After this, the kidneys and intestines were excised, weighed and sectioned for quantitative HA analysis or histochemical staining for HA as described below.

Water diuresis 4 h. Animals (rats n = 8; gerbils n = 10) received hypotonic glucose–saline solution after surgery. Urine was collected during eight consecutive 30 min sampling periods. After this, the kidneys (no intestines) were excised, weighed and sectioned for quantitative HA analysis or histochemical staining for HA as described below.

Water diuresis 6 h. Animals (rats n = 8; gerbils n = 7) received hypotonic glucose–saline solution after surgery. Urine was collected during 12 consecutive 30 min sampling periods. After this, the kidneys (no intestines) were excised, weighed and sectioned for quantitative HA analysis or histochemical staining for HA as described below.

HA content and distribution

The sectioning of kidneys to receive a specimen of the inner medulla (papilla) has previously been described in detail (Karlberg *et al.* 1983). Sectioning of the intestines into small specimens of duodenum (~1 cm from pylorus), jejunum (~10 cm from pylorus), ileum (~2 cm from the ileocaecal valve i.e. distal ileum) and proximal (~3 cm from the ileocaecal valve) and distal part of colon (~4 cm from rectum) were performed under a microscope at low magnification. Immediately after harvesting, specimens were put on filter paper and weighed 3 min later (wet weight, w.w.). They were lyophilized and then weighed again (dry weight, d.w.). After grinding, the HA was extracted from the tissues for 16 h with 0.5 M NaCl. Following centrifugation for 15 min at 2000 *g*, the HA content of the supernatants was analysed using a radiometric assay (Pharmacia Diagnostics, Uppsala, Sweden). The technique is based on the binding of HA to specific HA-binding proteins (Tengblad, 1980). Briefly, a 100 μl sample was incubated for 60 min at 4–7 °C with 200 μl ^{125}I-labelled HA-binding proteins. HA-Sepharose (100 μl) was added and the incubation continued for a further 45 min at the same temperature. Before centrifugation at 2000 *g* for 10 min, 2 ml washing solution was added. After decantation, the radioactivity in the pellet was measured in a gamma counter and a standard curve was constructed from samples with known amounts of HA. Double analyses were performed on each sample, with a variability of less than 10 %. The relative water content, expressed as a percentage of the total weight of the tissue, was calculated as 100(w.w. − d.w.)/w.w.

To determine the intrarenal distribution of HA, histochemical staining using an avidin-enzyme, biotin-protein system was performed. For this purpose the kidneys were stored in buffered 4 % formalin, pH 7.3, with 1 % cetylpyridiniumchloride (CPC) at room temperature until embedded in paraffin and sectioned. In brief, 4 μm thick sections were incubated with bovine serum albumin (10 mg ml^{-1}, Fraction V, Sigma Chemical Co., St Louis,

Table 1. Body weight, kidney weight and mean arterial blood pressure in rats and gerbils

				MAP (mmHg) selected periods of 30 min			
	Group	BW (g)	KW (g)	1	4	8	12
Rat	Vehicle	288 ± 10	2.14 ± 0.07	121 ± 7	119 ± 6	—	—
	2 h	290 ± 8	2.30 ± 0.09	131 ± 8	137 ± 9	—	—
	4 h	303 ± 17	2.23 ± 0.07	133 ± 5	130 ± 4	123 ± 5	—
	6 h	309 ± 23	2.37 ± 0.17	121 ± 4	120 ± 4	111 ± 4 *	101 ± 3 *
Gerbil	Vehicle	75 ± 3	0.60 ± 0.02	81 ± 3	66 ± 3 *	—	—
	2 h	77 ± 3	0.57 ± 0.03	72 ± 3	71 ± 4	—	—
	4 h	73 ± 2	0.58 ± 0.01	82 ± 5	67 ± 2 *	75 ± 3 *	—
	6 h	79 ± 3	0.64 ± 0.04	81 ± 1	77 ± 5	76 ± 3 *	78 ± 1 *

Body (BW) and kidney weights (KW, for both kidneys) and mean arterial blood pressure (MAP) in rats and gerbils subjected to I.V. water loading. * $P < 0.05$ *vs.* vehicle control in each species in MAP. This decrease is probably not due to the treatment but rather a common pattern observed in animals subjected to extended periods of anaesthesia.

MO, USA) to block non-specific binding sites and, thereafter, in 3 % H_2O_2 in PBS to inhibit endogenous peroxidase. After incubation for 2 h with biotinylated HA-binding proteins, the sections were incubated with ABC Vectastain Reagent (Vector Laboratories, Burlingame, CA, USA) for 1 h. Finally, H_2O_2 as substrate and 3-amino-9-ethyl-carbazole (AEC) as electron donor were added, and then the specimens were counterstained with Mayer's haematoxylin. Control sections were incubated for 2 h with *Streptomyces* hyaluronidase.

Statistical analysis

Data are given as mean values ± S.E.M. The comparison between groups was evaluated with Student's unpaired *t* test or ANOVA followed by Fisher's PLSD *post hoc* test when appropriate. A *P* value of less than 0.05 was considered significant.

RESULTS

Body weights, kidney weights and arterial blood pressures are given in Table 1. Blood pressure was slightly decreased during water loading in both rats and gerbils.

Tissue HA content

The renal papillary interstitial contents of HA in rats and gerbils are depicted in Fig. 1 (percentage change *vs.* control) and Table 2. In the rat, the amount of papillary HA increased during water loading. It was maximal after 2 h of water loading (+27 %, $P < 0.05$) and then declined to a level not significantly different from the controls. In the gerbil, the papillary HA content was only 37 % of that in the rat and a reverse effect of acute water loading on HA was found. Compared with control gerbils, papillary HA was reduced by 49 % ($P < 0.05$) after 2 h of water loading and was still reduced by 41 % ($P < 0.05$) after 6 h of water loading.

Along the intestine, the tissue levels of HA were similar in rats and gerbils (Fig. 2) and did not change during water loading (data not shown). The HA content in rats and gerbils was lowest in the duodenum and jejunum and about four times higher in the distal part of the colon.

Histochemical HA analysis

The histochemical visualization of HA in the rat kidney demonstrated positive staining in the papilla but no

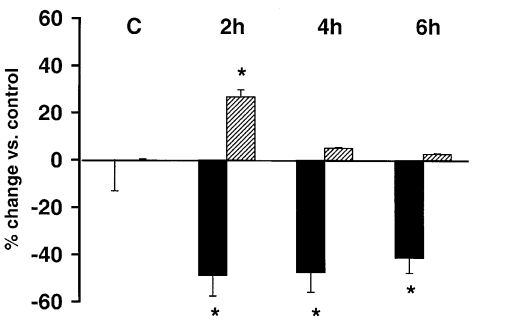

Figure 1. Percentage change in papillary HA in rats and gerbils

Percentage change in renal papillary HA content in control conditions (C), during up to 6 h of induced water diuresis in gerbils (filled bars) and in normal rats (hatched bars). * $P < 0.05$ *vs.* control for the respective species.

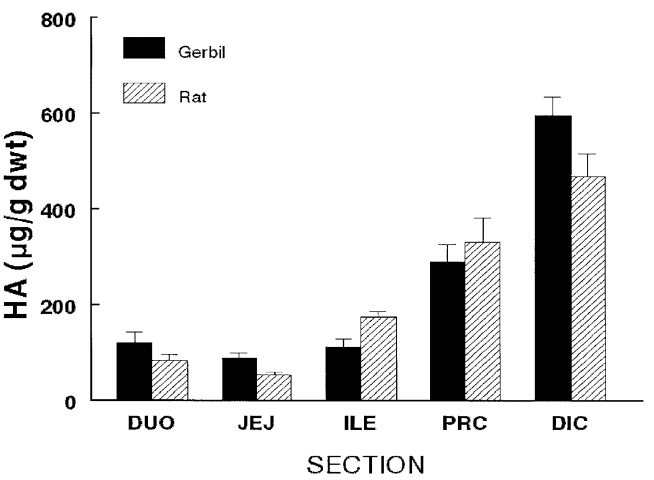

Figure 2. Intestinal HA in rats and gerbils

HA content along the intestines of gerbils (filled bars) and rats (hatched bars). Abbreviations: DUO, duodenum; JEJ, jejunum; ILE, distal ileum; PRC, proximal part of colon; DIC, distal part of colon.

Table 2. Papillary hyaluronan and urine osmolality in rats and gerbils

	Group	HA (μg(g d.w.)$^{-1}$)	Uosm (mosmol (kg H_2O)$^{-1}$)
Rat	Vehicle	413 ± 19	1659 ± 304
	2 h	559 ± 63 *	177 ± 64 *
	4 h	445 ± 39	194 ± 32 *
	6 h	435 ± 57	247 ± 80 *
Gerbil	Vehicle	156 ± 23	1910 ± 383
	2 h	80 ± 15 *	1379 ± 226
	4 h	82 ± 15 *	1494 ± 241
	6 h	92 ± 15 *	797 ± 218 *

Renal papillary interstitial HA and urine osmolality (Uosm) in rats and gerbils subjected to I.V. water loading. * $P < 0.05$ *vs.* vehicle control in each species.

staining in the interstitial tissue of the cortex and faint staining of the outer medulla. In gerbils, the staining was also mainly apparent in the papilla but with a different distribution, i.e. a more patchy pattern as compared to the rat (Fig. 3). No histochemical analysis was performed on intestinal tissue of either species.

Urine data

In the rat, the urine flow rate increased rapidly and peaked after 2 h (Fig. 4), i.e. during the peak elevation of papillary HA. The flow rate was 21 times higher after 2 h ($P < 0.05$) as compared with control levels and was 13 times higher after 6 h of water loading ($P < 0.05$). Urine osmolality (Table 2) decreased as expected during water loading and was lowest at peak urine flow rate (after 2 h) being 11 % of the control value ($P < 0.05$).

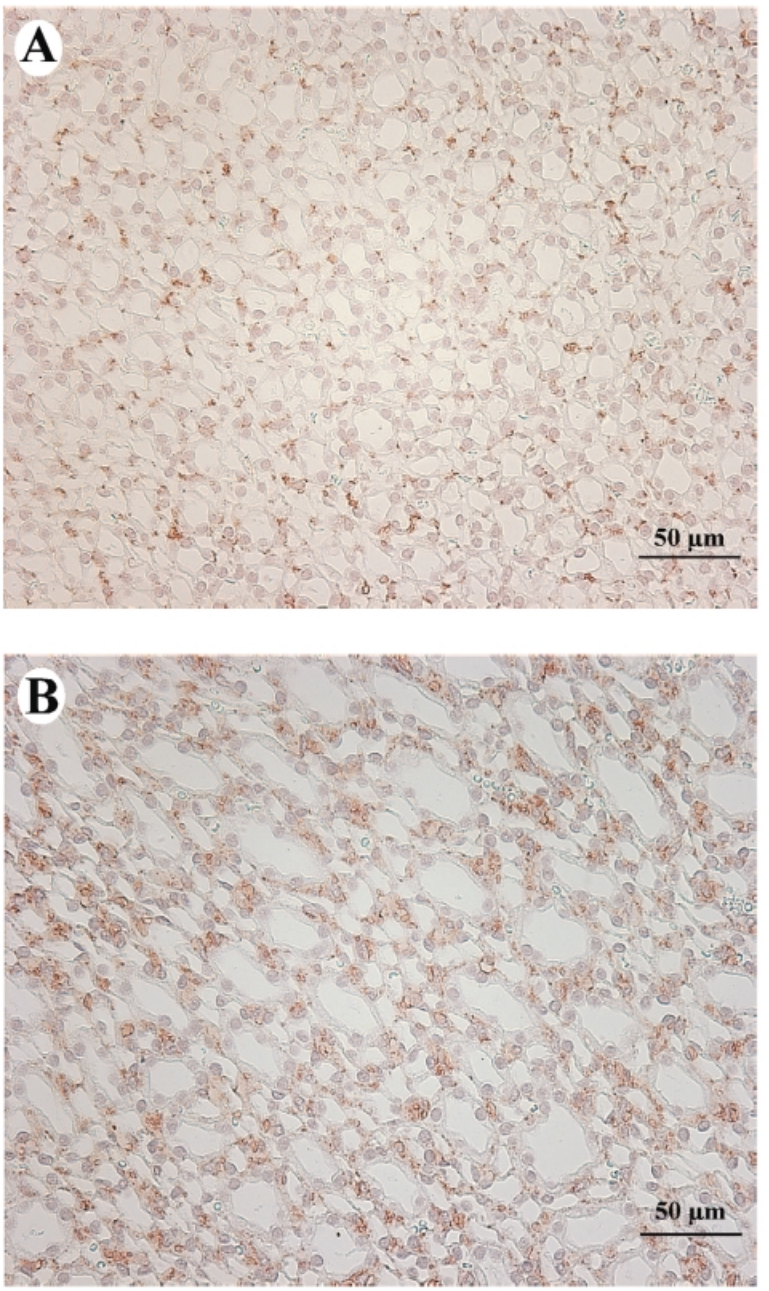

Figure 3. Histochemical distribution of HA in cross sections of renal papilla of gerbil (*A*) and rat (*B*)

The quantitative analysis revealed that gerbils only had about 37 % of the papillary HA content of the rat. Observe the more patchy appearance of HA staining in the gerbil as compared to that of the rat.

In the gerbil, the urine flow rate increased slowly and slightly with time and did not reach peak levels, which were six times higher than control levels, until after 6 h of water loading ($P < 0.05$). Urine osmolality tended to decrease after 2 h of water loading (n.s.) and was 42 % of the control value after 6 h, when the urine flow rate peaked ($P < 0.05$). The reduction in urine osmolality in the gerbil was less than that in the rat during water loading.

DISCUSSION

Our previous *in vitro* (Hansell *et al.* 1999; Göransson *et al.* 2001) and *in vivo* studies (Hansell *et al.* 2000) suggested that HA and renomedullary interstitial cells (RMICs) play an important role in the regulation of renal water handling in the rat. This conclusion was primarily based on findings in rats of an increased papillary HA content during acute water loading and decreased HA content during anti-diuresis. We also found that baseline papillary HA content of the gerbil was much lower than that of the normal rat. *In vitro* studies of rat RMICs, in an attempt to mimic the milieu of the renal medulla *in vivo* under conditions of water diuresis and dehydration, also supported the hypothesis. The results of the present study are in support of our previous studies suggesting an important role of renal HA as a complement to the powerful ADH system.

In the present study, we investigated the consequences of up to 6 h of I.V. water loading on papillary HA content in rats and gerbils. Our interest in this comparison of species emanates from the gerbil having an extreme ability to conserve water by producing concentrated urine. The low level of papillary HA in the gerbil would suggest a larger ability to reabsorb water. It is clear from the results of the present study that the gerbil has a completely different regulation of papillary HA from that of the rat: the elevation in papillary HA which occurs in the rat during acute water loading is diametrically changed into a decrease.

HA has an extreme capacity to bind water. This linear, negatively charged polysaccharide can form a unique network, which sequesters water molecules and forms a hydrated sphere (Laurent & Fraser, 1986). Studies on HA *in vitro* have shown that when the concentration of high molecular HA exceeds 0.1 mg ml^{-1}, inter- and intramolecular interactions occur such that the volume occupied by HA is increased, resulting in the exclusion of other macromolecules from their molecular environment. This phenomenon of steric exclusion may influence the osmotic activity and water transport in the intercellular matrix (Comper & Laurent, 1978). Thus, high HA concentrations in the renal papilla during water loading are likely to result in decreased water reabsorption from the medullary collecting system because HA can form a highly viscous solution around the collecting system which could change water transport possibilities. Structures of importance for water reabsorption are separated by the HA-rich matrix, which alters transport properties. Besides changing the transport distances, this 'functional oedema' may also change the interstitial hydrostatic pressure (Zawieja *et al.* 1992; Wang *et al.* 1998, 1999). As a consequence, the low papillary HA in the gerbil and further reduction during water loading would facilitate water reabsorption and thereby reduce the diuretic response.

The elevation of papillary HA which occurs within 2 h of water diuresis in the rat is in line with our previous investigation (Hansell *et al.* 2000). Furthermore, the present study shows that the elevation of HA in rats does not persist for 6 h, but rather tends to normalize through an unknown mechanism. It is interesting to note that the HA peak (+27 %) occurs simultaneously with the peak urine flow rate and that the latter levels out at 4 to 6 h of water loading about 10 times above control levels. It could be speculated that papillary HA in the rat is primarily important for the rapid and acute exclusion of water during excessive intake. For the gerbil, it is clear that the diametrically different response, i.e. the depression in papillary HA, does persist for at least the 6 h duration of the water loading and that the diuretic response is slow and relatively small in this period. It could thus be suggested that papillary HA in the gerbil is not only important during the acute phase of water loading as in the rat, but seems to be important for a longer period of water intake. Studies by McManus (1972) showed the gerbil's extreme ability to survive without water (12 days) but also the great ability of this species to rapidly rehydrate.

The mechanism underlying the elevation of papillary HA in the rat and the corresponding decrease in the gerbil during water loading is not known. Theoretically, it could be due to changed HA synthesis, changed HA degradation or a combination of both. As of now, we have no data on the behaviour of the HA synthases (HAS I–III), which have been demonstrated in the kidney (Spicer & McDonald, 1998; Feusi *et al.* 1999). We do, however, have indirect data

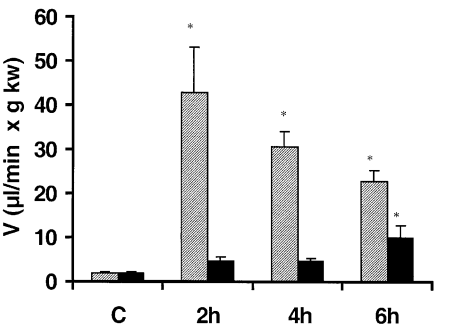

Figure 4. Urine flow rate in rats and gerbils

The urine flow rate (V) per gram kidney weight (kw) in rats (hatched bars) and gerbils (filled bars) in control conditions (C) and at the end of different time periods of induced water diuresis. * $P < 0.05$ *vs.* control in each species.

regarding the degradation. In our previous *in vitro* study (Göransson *et al.* 2001) on rat RMICs, we could demonstrate that the HA binding receptor CD44 is down-regulated during low medium osmolality, i.e. when the medium presence of HA increases, which could be translated to a situation of water loading. The opposite occurred during high medium osmolality (dehydration), i.e. increased presence of surface CD44 and reduced medium HA. The CD44 receptor has been shown to be involved in the turnover of HA through uptake and intracellular breakdown by acid hydrolysis in lysosomes, as has been shown for macrophages, cultured fibroblasts and chondrocytes (Culty *et al.* 1992; Hua *et al.* 1993). It could thus be speculated that the low papillary tissue osmolality which occurs during the induced water diuresis down-regulates surface CD44 receptors via an unknown mechanism. This, in turn, will reduce binding, internalization and breakdown of HA thus tending to elevate the interstitial HA.

The kidneys and the intestine are the two major organs involved in fluid transport. In the present study, we have focused on the changes occurring in papillary HA during control conditions and when the organism switches into a situation where excessive amounts of water are to be excreted. It is therefore interesting to note that high and low amounts of HA in different regions of both the kidney and the intestine correlate with the regions where small and large fluid volume transports occur. In the kidney, the major fluid volume reabsorption occurs in the cortex, where almost no HA is found. In the papilla, where less fluid volumes are reabsorbed, a very high amount of HA is detected. It is, however, important to acknowledge that the regulation of fluid balance occurs in the papilla (medulla) as opposed to the cortex although the papilla does not reabsorb the largest volume of fluid (Guyton & Hall, 1996). When making the same line of reasoning with the intestine it fits well with the pattern of the kidney: the lower levels of HA are found in the duodenum and jejunum where a large part of the fluid absorption takes place. The highest amounts of HA are found in the colon, which actually absorbs less fluid volumes although it stands for the final regulation of fluid absorption. The amount of HA in the intestines does not change in our model of induced water diuresis, i.e. during I.V. infusion of hypotonic saline. We believe, however, that giving similar amounts of fluid orally would be a better model to expose whether a similar change in HA levels occurs in the intestine as in the kidneys or whether a different HA regulation is at hand. It is also noteworthy that the major species difference in HA observed in the renal papilla is not found in the intestines, where quite similar levels are detected. This might be explained by the healthy intestine absorbing practically all the water ingested and that the main function of the gastrointestinal tract in water homeostasis is to deliver water to the systemic circulation (Turnheim, 1984). Renal mechanisms, on the other hand, fulfil the function of preserving the dynamic water equilibrium of the whole organism.

Gerbils and rats differ in several respects. Regarding the kidney, there are anatomical and physiological differences (Schmidt-Neilsen, 1964; Buchanan & Stewart, 1974; Natochin *et al.* 1983). Regarding the renomedullary interstitial cells, RMICs, there are also species-related differences. This cell type is found in the medulla and its most characteristic feature is the abundance of lipid droplets. RMICs are believed to provide structural support to the renal tubules and blood vessels but also to be involved in the regulation of renal blood flow and urine concentration, possibly involving the lipid found in the lipid droplets. In water-loaded rats that excreted urine of relatively low osmolality, the number of lipid droplets in the RMICs was two times larger than in untreated rats. When similar studies were conducted in gerbils, it was found that the amount of lipid droplets decreased after water loading (Bohman & Jensen, 1978). ADH is naturally an important factor, which translocates aquaporins from intracellular vesicles into the apical membrane of the distal tubule and collecting system (Nielsen *et al.* 1993), leading to an increased ability to reabsorb water. We suggest that the papillary HA system works as a complement to regulate water permeability and thereby participates in the regulation of fluid homeostasis. Regarding the levels of ADH, these are four times higher in the gerbil than in the rat under control conditions (Baddouri & Quyuo, 1991; Huang *et al.* 1994). This could explain the differences in HA between the two species observed under normal conditions, since ADH has been shown to activate the HA degrading enzyme hyaluronidase (Ivanova & Melidi, 1999). Differences in ADH handling have been studied: during long times of water deprivation, the ADH depletion is smaller in the gerbil, thereby suggesting that the synthesis ability is greater (Donaldson & Edwards, 1981). In a study of the activity of the hypothalamus–neurohypophysis during rehydration, Edwards (1984) provided further evidence of this by showing that water deprivation for 3 or 5 days resulted in the same degree of ADH depletion but that the ADH stores were replenished much faster in the gerbil. Gerbils produced small volumes of more concentrated urine even 24 h after free access to water, while the rat produced urine of similar volume and concentration as the control animals. This might point to the fact that gerbils have a higher plasma concentration of ADH, even after water deprivation (the rehydration phase).

In conclusion, in the rat, the elevation of papillary interstitial HA that occurs in response to acute water loading would counteract water reabsorption by changing the physico-chemical characteristics of the interstitial matrix favouring water diuresis. In combination with

reduced levels of ADH-regulated aquaporins, this would result in a rapid and major diuretic response. The gerbil has a diametrically different regulation of papillary HA turnover during water loading. The decreased papillary HA level in gerbils during water loading and the slow and small diuretic response may represent a genetic difference in adaptation in this species to ensure the ability to conserve water in an arid environment.

REFERENCES

Badduori, K. & Queue, A. (1991). Osmolality and secretion of vasopressin during pregnancy in *Meriones crassus*. *Reproduction Nutrition Development* **31**, 535–539.

Bohman, S.-O. & Jensen, K. A. (1978). The interstitial cells in renal medulla of rat, rabbit and gerbil in different states of diuresis. *Cell Tissue Research* **189**, 1–18.

Buchanan, J. G. & Stewart, A. D. (1974). Neurohypophysial store of vasopressin in the normal and the dehydrated gerbil (*Meriones unguiculatus*) with a note on kidney structure. *Journal of Endocrinology* **60**, 381–382.

Comper, W. D. & Laurent, T. C. (1978). Physiological function of connective tissue polysaccharides. *Physiological Reviews* **58**, 255–315.

Culty, M., Nguyen, H. A. & Underhill, C. B. (1992). The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. *Journal of Cell Biology* **116**, 1055–1062.

Donaldson, K. & Edwards, B. (1981). A comparison of the effects of saline imbibtion and water deprivation on the composition of the blood, urine and pituitary neural lobe of the gerbil (*Meriones unguiculatus*). *Comparative Biochemistry and Physiology* A **68**, 31–35.

Edwards, B. A. (1984). The activity of the hypothalamo-neurohypophysis during rehydration following water deprivation in the gerbil (*Meriones unguiculatus*) and the laboratory rat (*Rattus norwegicus*). *Comparative Biochemistry and Physiology* A **77**, 557–561.

Feusi, E., Sun, L., Sibalic, A., Beck-Schimmer, B., Oertli, B. & Wuthrich R. P. (1999). Enhanced hyaluronan synthesis in the MRL-Fas(lpr) kidney: role of cytokines. *Nephron* **83**, 66–73.

Fraser, J. R. E. & Laurent, T. C. (1989). Turnover and metabolism of hyaluronan. In *The Biology of Hyaluronan,* Ciba Foundation Symposium 143, ed. Evered, D. & Whelan, J., pp. 41–59. Wiley, Chichester, UK.

Gerdin, B. & Hällgren, R. (1997). Dynamic role of hyaluronan in connective tissue activation and inflammation. *Journal of Internal Medicine* **242**, 49–55.

Ginetzinsky, A. G. (1958). Role of hyaluronidase in the reabsorption of water in renal tubules: the mechanism of action of the antidiuretic hormone. *Nature* **182**, 1218–1219.

Göransson, V., Hansell, P., Moss, S., Alcorn, D., Johnsson, C., Hällgren, R. & Maric, C. (2001). Renomedullary interstitial cells in culture; the osmolality and oxygen tension influence the extracellular amounts of hyaluronan and cellular expression of CD44. *Matrix Biology* **29**, 129–136.

Guyton, A. C. & Hall, J. E. (1996). The kidneys and body fluids. In *Textbook of Medical Physiology*, 9th edn, pp. 297–421. W. B Saunders, Philadelphia.

Hansell, P., Göransson, V., Odlind, C., Gerdin, B. & Hällgren, R. (2000). Hyaluronan content in the kidney in different states of body hydration. *Kidney International* **58**, 2061–2068.

Hansell, P., Maric, C., Alcorn, D., Göransson, V., Johnsson, C. & Hällgren, R. (1999). Renomedullary interstitial cells regulate hyaluronan turnover depending on growth media osmolality suggesting a role in renal water handling. *Acta Physiologica Scandinavica* **165**, 115–116

Hällgren, R., Gerdin, B. & Tufveson, G. (1990). Hyaluronic acid accumulation and redistribution in rejecting rat kidney graft: relationship to the transplantation oedema. *Journal of Experimental Medicine* **171**, 2063–2076.

Hua, Q., Knudson, C. B. & Knudson, W. (1993). Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. *Journal of Cell Science* **106**, 365–375.

Huang, W., Lee, S.-L. & Sjöqvist, M. (1994). Effects of neuorhypophyseal antagonists in postnephrectomy natriuresis in male rats. *Kidney International* **45**, 692–699.

Ivanova, L. N. & Melidi, N. N. (1999). Hyaluronate-hydrolases system and hydroosmotic effect of vasopression. *Rossiiski fiziologicheskii zurnal imeni I. M. Sechenova/Rossiiski academia nauk* **85**, 847–856.

Johnsson, C., Tufveson, G., Wahlberg, J. & Hällgren, R. (1996). Experimentally induced warm renal ischemia induces cortical accumulation of hyaluronan in the kidney. *Kidney International* **50**, 1224–1229.

Karlberg, L., Norlén, B. J., Öjteg, G. & Wolgast, M. (1983). Inpaired medullary circulation in postischemic acute renal failure. *Acta Physiologica Scandinavica* **118**, 11–17.

Laurent, T. C. & Fraser, J. R. E. (1986). The properties and turnover of hyaluronan. In *Functions of the Proteoglycans*, Ciba Foundation Symposium 124, ed. Evered, D. & Whelan, J., pp. 9–29. Wiley, Chichester, UK.

Law, R. O. & Rowen, D. (1981). The role of hyaluronidase on urinary and renal medullary composition following anti diuretic stimulus in the rat. *Journal of Physiology* **311**, 341–354.

Lewington, A. J. P., Padanilam, B. J., Martin, D. R. & Hammerman, M. R. (2000). Expression of CD44 in kidney after acute ischemic injury in rats. *American Journal of Physiology – Renal Physiology* **278**, R247–254.

McManus, J. (1972). Water relations and food consumption of the mongolian gerbil, *Meriones unguiculatus*. *Comparative Biochemistry and Physiology* A **43**, 959–967.

Natochin, I. V., Dlouha, H, Lavrova, E. A., Pechurkina, N. I. & Podsekaeva, N. I. (1983). The osmoregulating function of the kidney and activity of the loop Henle in water rats, gerbils and Brattleboro rats. *Fiziologicheskii zhurnal imeni I. M. Sechenova* **69**, 695–700.

Nielsen, S., DiGiovanni, S. R., Christensen, E. I., Knepper, M. A. & Harris, H. W. (1993). Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. *Proceedings of National Academy of Sciences of the USA* **90**, 11663–11667.

Schmidt-Nielsen, K. (1964). *Desert animals. Physiological Problems of Heat and Water*. Oxford University Press, Oxford.

Spicer, A. P. & McDonald, J. A. (1998). Characterization and molecular evolution of a vertebrate hyaluronan synthase gene family. *Journal of Biological Chemistry* **273**, 1923–1932.

Tengblad, A. (1980). Quantitative analysis of hyaluronate in nanogram amounts. *Biochemical Journal* **185**, 101–105.

Turnheim, K. (1984). Intestinal permeation of water. In *Handbook of Experimental Pharmacology*, ed. Csaky, T. Z., pp. 381–463. Springer, New York.

WANG, T., CHENG, H. H., HEIMBURGER, O., WANIEWSKI, J., BERGSTRÖM, J. & LINDHOLM, B. (1998). Hyaluronan prevents the decreased net ultrafiltration caused by increased peritoneal dialysate fill volume. *Kidney International* **53**, 496–502.

WANG, T, CHENG, H. H., HEIMBURGER, O., WANIEWSKI, J., CHEN, C., BERGSTRÖM, J. & LINDHOLM B. (1999). Hyaluronan decreases peritoneal fluid absorption: Effect of molecular weight and concentration of hyaluronan. *Kidney International* **55**, 667–673.

WU, C.-M. & SHEN, C.-L. (1994). The distribution of vasopressinergic and oxotocinergic neurons in the CNS of the gerbil. *Zoological Studies* **33**, 114–125.

ZAWIEJA, D., GARCIA, C. & GRANGER, H. (1992). Oxygen radicals, enzymes and fluid transport through pericardial interstitium. *American Journal of Physiology* **262**, H136–143.

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